Stability of DON and DON-3-glucoside during baking as affected by the presence of food additives

Arnau Vidal, Vicente Sanchis, Antonio J. Ramos and Sonia Marín*

Food Technology Dept, XaRTA-UTPV, Agrotecnio Center, University of Lleida, Spain

*Corresponding author: Tel. +34-973-702542. Fax: +34-973-702596. E-mail address: smarin@tecal.udl.cat. Postal address: Avda. Rovira Roure 191, 25198 Lleida, Spain

Abstract

The mycotoxin deoxynivalenol (DON) is one of the most common mycotoxins of cereals worldwide, and its occurrence has been widely reported in raw wheat. The free mycotoxin form is not the only danger of mycotoxin exposure; modified forms can also be present in cereal products. Deoxynivalenol-3-glucoside (DON-3-glucoside) is a common DON plant conjugate. The mycotoxin concentration could be affected by food processing; here, we studied the stability of DON and DON-3-glucoside during baking of small doughs made from white wheat flour and other ingredients. A range of common food additives was added to assess possible interference: ascorbic acid (E300), citric acid (E330), sorbic acid (E200), calcium propionate (E282), lecithin (E322), diacetyltartaric acid esters of fatty acid mono- and di-glycerides (E472a), calcium phosphate (E341), disodium diphosphate (E450i), xanthan gum (E415), polydextrose (E1200), sorbitol (E420i) and sodium bicarbonate (E500i) as well as wheat gluten and malt flour. The DON content was reduced by 40 %, and DON-3-glucoside concentration increased by > 100 %, after baking for 20 minutes at 180 °C, this confirmed that

DON and DON-3-glucoside concentrations can vary during heating and even DON-3-glucoside could increase after baking. However, DON and DON-3-glucoside are not affected significantly by the presence of the food additives tested.

Keywords: deoxynivalenol, deoxynivalenol-3-glucoside, baking, additives

Highlights:

- Deoxynivalenol is not affected by common additives during baking.
- Deoxynivalenol-3-glucoside is not affected during baking due to additive presence.
- There is a possibly high increase of deoxynivalenol-3-glucoside due to baking.

1. Introduction

Deoxynivalenol (DON) is a trichothecene mycotoxin produced by fungi of the *Fusarium* genus, for example *F. culmorum* and *F. graminearum*. Environmental conditions that favour DON production in the field are low temperature and high humidity. Cold and wet weather, which tends to delay harvest, permits continued growth of mould on the crop, thereby increasing the chance of higher concentrations of DON being produced (SCF 1999).

In general, acute exposure of animals to DON can result in decreased feed consumption (anorexia), vomiting (emesis), abdominal pain, dizziness, headache, throat irritation, diarrhoea, and blood in stool (Rotter et al. 1996). While longer exposure can cause reduced growth, and adverse effects on the thymus, spleen, heart, and liver (Sobrova et al. 2010). Monogastric animals, especially swine, show the greatest sensitivity to DON, while chickens and turkeys, followed by ruminants, appear to have higher tolerance (Rocha et al. 2005). Concerning human safety, Scientific Committee on Food (SCF) established a temporary Tolerable Daily Intake of 1 µg/kg body weight per day.

DON occurs predominantly in grains such as wheat, barley, oats, rye and maize, and less often in rice, sorghum and triticale. Thus, DON is found in cereal-based foods (Cano-Sancho, Valle-Algarra, et al., 2011) and feedstuffs (Döll and Dänicke 2011) and this type of food is considered as the major contributor of human intake of DON (Cano-Sancho, Gauchi, et al. 2011). Due to the high presence of DON in cereals, different maximum levels exist for DON in unprocessed

cereals and processed cereals/cereal-based foods (e.g. 1250 µg/kg for unprocessed common wheat and 500 µg/kg for bread and bakery wares) (EC 2006).

Moreover, wheat grains with presence of DON may also contain deoxynivalenol-3-glucoside (DON-3-glucoside), a metabolite of DON produced by plants (Berthiller et al. 2009). The reported levels of DON-3-glucoside are variable, however, the concentration ratio DON-3-glucoside/DON is described to range from around 10 to 30 % (Berthiller et al. 2009; Dall'Asta et al. 2013). Moreover the presence of DON-3-glucoside in processed cereal products has been also confirmed (Suman et al., 2013). Furthermore, Berthiller et al. (2011) showed that DON-3-glucoside can be hydrolysed to DON by several lactic acid bacteria that may be present in the intestine. Therefore, the FAO/WHO Expert Committee (JECFA) has considered DON-3-glucoside as an additional contributing factor of the total dietary exposure to DON (JECFA 2010).

Processing of cereals at high temperatures may affect the DON and DON-3-glucoside content but thermal food processing seems to be quite variable and dependent on the processing conditions applied: the temperature, the baking time, the type of mycotoxin, the ingredients, and the size of cereal product. Some studies have reported a significant decrease in the DON level during baking of bakery products (Valle-Algarra et al. 2009; Numanoglu et al. 2012). In contrast, studies by De Angelis et al. (2013) and Zachariasova et al. (2012) have reported that DON is stable during processing steps involving high temperatures. The contradictory published results are sometimes due to the different sizes of assayed products, which affect the heat transfer and favours temperature gradients inside the products (Vidal et al. 2015). Moreover, the possible release of DON from the flour could be due to the presence of some added enzymes (Vidal, Ambrosio, et al. 2016). Similar to DON, DON-3-glucoside stability is also affected by the different size of the product, which has caused variable results in past studies (Suman et al. 2012; Zachariasova et al. 2012; Vidal, Marín, et al. 2014; Vidal, Morales,

et al. 2014; Generotti et al., 2015). However, Vidal et al. (2015) showed that DON-3-glucoside may be released under mild baking conditions of temperature and time (for instance, 140 °C for 35 minutes or 200 °C for less than 10 minutes) but reduced under harsher baking conditions (i.e., longer periods of time and higher temperatures).

The current study aimed to investigate the effect of food additives or ingredients commonly used in the food industry (ascorbic acid (E300), citric acid (E330), sorbic acid (E200), calcium propionate (E282), lecithin (E322), diacetyltartaric acid esters of mono and diglycerides of fatty acids (E472a), calcium phosphate (E341), disodium diphosphate (E450i), xanthan gum (E415), polydextrose (E1200), sorbitol (E420i), sodium bicarbonate (E500i), gluten of wheat and malt flour) on DON and DON-3-glucoside stability during baking.

2. Materials and Methods

2.1. DON and DON-3-glucoside contaminated flour

Wheat flour was purchased in a flour mill in Lleida (Spain), and analysed for natural DON and DON-3-glucoside contamination.

To obtain a higher concentration of DON in the contaminated flour, a strain of *Fusarium graminearum* (TA 3.234) was used. It is maintained in the Food Technology Dept. collection at the University of Lleida in Spain. It was previously proven to be DON producer when cultured on wheat flour. The strain was inoculated and incubated in MEA (malt extract agar) at 25 °C until vigorous sporulation occurred. A spore suspension of the mould was prepared in water and Tween 80 (0.005 % v/v). Five millilitres of a *F. graminearum* spore suspension were inoculated in a glass flask containing 250 g of flour and 50 mL of water. In total, 3 kg of flour

were inoculated with the strain. The flasks were stored at 25 °C for 19 days with periodic shaking. Then, the content of the flasks was powdered and homogenized and subjected to a DON analysis. The content of DON was of 12,500 ± 1,235 µg/kg (n=3). The concentration of DON-3-glucoside (a plant metabolite) did not change.

2.2. Bakery analogue preparation

First, the flour used was previously prepared by mixing uninoculated flour with DON contaminated flour. The concentrations of DON and DON-3-glucoside were 1108 ± 55 and 62 ± 14 µg/kg (n = 5), respectively. A bakery product analogue was prepared for each 100 g of mixture with 27 g of wheat flour, 26 g of sucrose, 26 g of eggs, 21 g of sunflower oil and 0.5 g of baking powder containing maize starch, sodium bicarbonate and disodium diphosphate was added to 100 g of the mixture. In addition to the described ingredients, we added a different additive/ingredient to the mixture to produce each treatment or no additive/ingredient was added to the mixture to provide a control. We did three repetitions for each additive (Table 1). The additives and their quantity were selected after consulting information for bakery industry, as well as scientific references. Thus, the additives tested are regularly used in the bakery industry: ascorbic acid (E300) (> 99 %, Merck, Madrid, Spain), citric acid (E330) (> 99.5 %), sorbic acid (E200) (> 98.5 %), calcium phosphate (E341) (> 98 %) disodium diphosphate (E450i) (> 98 %) all of them purchased from Panreac (Castellar del Valles, Spain), calcium propionate (E282), diacetyltartaric acid esters of mono and diglycerides of fatty acids (E472a) (> 99 %), xanthan gum (E415) (> 99 %), polydextrose (E1200) (> 99 %), sorbitol (E420i) (> 98 %), sodium bicarbonate (E500i) (> 99 %) and lecithin (E322) (> 99 %) all of them purchased from Sigma-Aldrich (Alcobendas, Spain). Further wheat gluten (El Granero Integral, Madrid, Spain) and malt flour (Puratos, Girona, Spain) were also tested.

The mixture was manually mixed and 20 g aliquots were poured into small paper moulds. The pH of each mix was measured with a pH meter (Crison MicropH 2001, Crison Instruments SA, Alella, Spain). From this point, thermoprobes (Proges Plus, Pluck&Track, Thermo bouton) were always used in the bakery matrixes to register the baking temperature. The probes were placed in the centre of the mould before placing them in the oven at 180 °C for 20 minutes. These conditions were established on the basis of previous experiments and are commonly used conditions in the baking industry. After baking, all samples were lyophilised for 72 h, and the samples were stored at –20 °C until analysis.

2.3. Preparation of mycotoxin standard solutions

A DON standard (Sigma-Aldrich, Alcobendas, Spain) solution was dissolved in ethanol (Sigma-Aldrich, Alcobendas, Spain) at a concentration of 10.0 µg/mL and stored at 4 °C. The concentration in the stock solution was checked by UV spectrometry according to the AOAC Official methods of analysis. Working standards (2.5, 1.0, 0.5, 0.1 and 0.05 µg/mL) were prepared by the appropriate dilution of known volumes of the stock solution with the mobile phase and used to obtain calibration curves in an appropriate chromatographic system. A DON-3-glucoside standard (Sigma-Aldrich, Alcobendas, Spain) was dissolved in acetonitrile (J.T. Baker, Deventer, The Netherlands) at a concentration of 10.0 µg/mL and stored at 4 °C in a sealed vial until use. Working standards (1.0, 0.5, 0.1, 0.05 and 0.01 µg/mL) were prepared as for DON, and the calibration curves were generated in a similar fashion.

2.4. Mycotoxins extraction, detection and quantification

DON and DON-3-glucoside were extracted from 5 g of a lyophilised ground sample (IKA® A11B basic analytical mill, IKA-Werke GmbH & Co. KG, Germany) with 30 mL of distilled water with magnetic stirring for 10 min. The sample was centrifuged for 8 min at 1780

148 *g*. The supernatant was filtered through a filter paper (Whatman No. 1, Whatman, Maidstone,
149 UK). Five millilitres of filtered sample were loaded on a DONPREP® IAC column (R-
150 Biopharm, Rhone LTD Glasgow, UK) and the column was washed with 10 mL of distilled
151 water. DON and DON-3-glucoside were eluted by consecutively applying 1.5 mL of methanol
152 grade HPLC (Sigma-Aldrich, Alcobendas, Spain) (with three backflushing steps) and 1.5 mL
153 of milli-Q water (Millipore, Billerica, MA, USA). Zachariasova et al. (2012) confirmed that
154 DON-3-glucoside had good cross-reactivity with the IAC DONPREP® columns (99-102 %
155 recovery for DON and DON-3-glucoside when less than 500 ng of these toxins were loaded).
156 The purified extracts were dried under a nitrogen stream at 40 °C. Each dried sample was
157 resuspended in 0.5 mL of the mobile phase (water:acetonitrile:methanol, 92:4:4). DON and
158 DON-3-glucoside were determined in an HPLC Waters 2695® system, with a Waters
159 Spherisorb® 5 µm ODS2, 4.6 x 250 mm analytical column coupled with a Waters 2487
160 UV/Visible dual λ absorbance Detector. The absorption wavelength was set at 220 nm. The
161 HPLC mobile phase flow rate was 0.6 mL/min, the injection volume was 100 µL, the column
162 temperature was 40 °C and the retention times for DON and DON-3-glucoside were 20 and 23
163 min, respectively.

164

165 *2.5. Methods performance*

166 The analytical methods for DON and DON-3-glucoside were assessed for linearity,
167 precision and recovery with spiked flour and baked products. The possible interference of
168 additives used in the methods performance was not evaluated. Standard curves were generated
169 by the linear regression of the peak areas against concentration (the r^2 values were 0.98 and 0.97
170 for DON and DON-3-glucoside, respectively). The precision was established by determining
171 the DON and DON-3-glucoside levels in the flour and the DON in the bakery product analogue

samples in triplicate at least, in fortified samples so the recovery rates could be calculated. The limit of detection (LOD) was considered to be three times the signal of the blank reading, and the limit of quantification (LOQ) was set as 3 x LOD. The method performance characteristics for DON and DON-3-glucoside are summarized in Table 2.

2.6. Statistics

The results are given on a dry weight basis and they were not corrected by recovery values. It is worthy to mention that the mycotoxin concentration was not corrected according to the recipe formulation, because the percent contribution of the additives/ingredients was small. Three completely independent repetitions were made for each additive treatment. An ANOVA was used to assess the significance of the sample parameters (additive or ingredient additions) in the observed mycotoxin concentrations as well as in the calculated increase or reduction percentages. Correlation analysis was applied in order to check the relation between pH and mycotoxin variation. The software used for statistical analysis was Microsoft Excel® (Microsoft® Corporation, California) and Statistics 20.0 (IBM SPSS® Statistics 20.0 Inc., Chicago).

3. Results and Discussion

3.1. Initial levels of DON and DON-3-glucoside in flour

The initial DON and DON-3-glucoside concentrations in the natural flour (n = 5) were 252 ± 30 and 60±15 µg/kg, respectively. However, an aliquot of the wheat flour was uninoculated with a *Fusarium* mould to get more DON in the flour, to get higher levels to be able to see clear differences in the results. For instance, Stanciu et al. (2017) found an average

DON concentration of 190 µg/kg in wheat flour from Romania with a maximum concentration of 1787 µg/kg or Cano-Sancho et al. (2011b) found an average DON concentration of 739 µg/kg. Other studies showed more worrying DON concentrations in cereal based products and even DON concentrations over the maximum DON concentration set up by the EC have been found (SCOOP 2003; Cano-Sancho, Gauchi, et al. 2011; Vidal et al. 2013).

Unluckily the DON-3-glucoside concentration could not be modified, because it is formed by the plant. The presence of DON-3-glucoside in raw cereals is also common. Several studies showed the co-occurrence of the two mycotoxins in raw products and the concentration ratio DON-3-glucoside/DON is described to range from around 10 to 30 % (Berthiller et al. 2009; Dall'Asta et al. 2013) as in our flour (ratio DON-3-glucoside/DON = 24 %).

The mycotoxins levels found in flour showed that despite all the effort to control mycotoxins presence through the food chain, DON and its modified forms still have a wide incidence in wheat products. Moreover, the high levels of DON in cereals cause cereal based food to be the main source of exposure to this mycotoxin (Marin et al. 2013).

3.2. DON stability

The uncooked food matrix had a DON concentration of 350 ± 43 µg/kg. The DON concentration in the flour was 1108 ± 55 µg/kg but mixing the ingredients reduced the DON by 68 % due to dilution by the flour. A DON concentration reduction by 42 % was achieved in control samples after baking for 20 min at 180 °C and an average DON concentration of 201 ± 28 µg/kg was found. On the other hand, the added ingredients and additives did not cause a significant change in the DON concentration compared to the control ($p > 0.05$, ANOVA test).

Due to the high prevalence of DON in cereal-based products (Marín et al. 2013), the stability of DON during cereal processing has been deeply studied, however there is a high

variability of results among the published studies. Baking caused a reduction of the DON in the baked products by 42 %. Mycotoxins are considered highly thermostable compounds, however, they can be reduced during baking, but many factors affect their final concentration. For this reason, existing studies are contradictory regarding DON fate during baking (Neira et al. 1997; Bergamini et al. 2010; Zachariasova et al. 2012; Vidal, Morales, et al. 2014). Product size is a very important factor. Small products as used in this study allow for rapid heat transmission (Vidal et al. 2015). On the other hand, the temperature inside bread loaves is typically below 100 °C, regardless of the baking temperature (Vidal, Morales, et al., 2014), differently than for our products, for which higher temperatures were registered (Figure 1). The presence of enzymes could affect the stability of DON, for example, xylanase and α-amylase produced increases of DON after baking (Vidal, Sanchis, et al. 2016; Vidal et al. 2017), because the enzymes released DON embedded in the wheat matrix.

Additives added to the products did not produce changes ($p > 0.05$, ANOVA test) in DON concentration compared to the control and a similar reduction was observed (Table 1). Additives such as sorbitol or ascorbic acid are used by the food industry for example to improve flavour or to extend the shelf life of bakery products. Some of them can change the pH of the food (e.g., sorbic acid or citric acid), and DON is sensitive to pH variations. Some additives caused pH decreases until pH = 5.2 (ascorbic acid). However, the pH variation was not correlated with the reduction percentage. Mishra et al. (2014) showed that the DON concentration declined sharply at pH = 3, and it was much lower than the values recorded in this study. Chang et al. (2015) did not find differences in the final DON concentration during the bread-making process in the presence of L-cysteine hydrochloride (E920). Boyacioglu et al. (1993) examined the effect of potassium bromate (E924) and ascorbic acid (E300) and these additives did not change the DON concentration during the bread-making process compared to breads without additives. On the other hand, they detected reductions of up to 40 % due to the

presence of an additive compared to the breads without an additive when they used sodium bisulphite (E222), l-cysteine (E920) and ammonium phosphate (E342i). However, European legislation does not permit the use of ammonium phosphate (E342). Sodium bisulphite (E222) and l-cysteine (E920) cause the breakage of disulphide bonds, which are covalent bonds between the sulphur atoms of two molecules of the amino acid cysteine. Therefore, DON could be linked to these covalent bonds in the wheat matrix.

Finally, a DON reduction may cause the appearance of DON reduction products. Mishra et al. (2014) and Vidal et al. (2015) observed the formation of deepoxy-deoxynivalenol as a degradation product of DON after heating at 125 – 200 °C. Moreover, Bretz et al. (2006) detected other degradation products after heating DON, including norDON A, B and C, but a lack of standards for these products did not allow their quantification in this assay.

3.3. DON-3-glucoside stability

The DON-3-glucoside concentration was below the LOD (14 µg/kg) in the uncooked food matrix. Although the initial DON-3-glucoside concentration was 60 µg/kg, dilution by the other ingredients caused a reduction of the DON-3-glucoside concentration. It would be interesting to try to get a larger DON-3-glucoside concentration in the initial wheat flour, but as it is formed by plants, it is not possible to modify its concentration in the flour, without direct spiking. However, we were able to detect DON-3-glucoside in the baked products because it increased during the baking step. Hence, the DON-3-glucoside concentration in the control baked product was 57 µg/kg. So, baking produced a DON-3-glucoside increase compared with the DON-3-glucoside concentration in the uncooked mix (< LOD).

DON-3-glucoside is a conjugated DON mycotoxin produced by the plant as a detoxification product in which the hydroxyl group in position C13 of DON is replaced by a

glucose unit. DON-3-glucoside in cereal grains has been known for years. Sewald et al. (1992) were the first to detect this DON metabolite in maize plants. Assays show a high percentage of DON-3-glucoside in some crops, and even all the analysed cereal samples (wheat, maize, oat and triticale) from some studies had DON-3-glucoside presence (Berthiller et al. 2009; Desmarchelier and Seefelder, 2010; Rasmussen et al. 2010; Dall'asta et al. 2013), showing the high presence of this modified mycotoxin form. Because of its high prevalence in cereals, the stability of DON-3-glucoside during baking has been previously studied.

The observed increase of DON-3-glucoside after baking agrees with previous similar studies in which DON-3-glucoside increased (Vaclavikova et al. 2013; Vidal, Marin, et al. 2014; Vidal et al. 2015); however, some studies have shown a reduction in DON-3-glucoside after baking (Kostelanska et al. 2011; Simsek et al. 2012). The presence of enzymes (e.g., xylanase, amylase, cellulase, protease and glucose-oxidase) (Vidal, Ambrosio, et al. 2016), the product size, the temperature and the baking time (Vidal et al. 2015) seem to be important factors for the stability of DON-3-glucoside during baking. Therefore, mild baking conditions (for instance, 140 °C for 35 minutes or 200 °C for less than 10 minutes) can cause increases of DON-3-glucoside at the end of baking while harsher conditions (i.e., longer baking times and higher temperatures) lead to a reduction in DON-3-glucoside as Vidal et al. (2015) obtained in their results. Therefore, the increase of DON-3-glucoside in our study was expected, because we used mild baking conditions. This increment in DON-3-glucoside level and DON reduction during baking has been previously demonstrated because the concentration ratio DON-3-glucoside/DON is described to range from around 10 to 30 % in raw cereals (Berthiller et al. 2009; Desmarchelier et al. 2010, Rasmussen et al. 2010; Dall'asta et al. 2013), while this ratio in baked products increases and even in some cases the DON-3-glucoside concentration is the same than DON concentration (De Boevre et al. 2013). Thus the DON-3-glucoside increase and the possible DON reduction during baking cause the ratio increase. Kostelanska et al.

293 (2011) suggested that DON-3-glucoside the link to the cellular polysaccharides of the matrix in
294 raw products is through glycoside bonds. This attachment of DON-3-glucoside to the matrix
295 causes the no detection of DON-3-glucoside during analysis of raw products. Then, food
296 processing breaks the glycoside bond and it brings about the detected increase of DON-3-
297 glucoside in the processed products.

298 More studies that examine the relationship between DON and DON-3-glucoside are
299 necessary to fully understand the interactions between the parent and conjugated mycotoxins in
300 food processing.

301 Additives did not affect the DON-3-glucoside concentration after baking (Table 1, ($p >$
302 0.05, ANOVA test). Unfortunately, other studies on DON-3-glucoside stability in the presence
303 of additives do not exist.

304 Finally, the increase in DON-3-glucoside during baking is of concern. In spite of the
305 fact that in this study the initial DON-3-glucoside concentration is low and consequently its
306 final concentration in the cooked matrix is not high, the large increase of DON-3-glucoside
307 concentration during baking could be more problematic when the initial DON-3-glucoside
308 concentration is higher. On the other hand, although DON-3-glucoside is far less active as an
309 inhibitor of protein biosynthesis than DON (Poppenberger et al. 2003), DON-3-glucoside is
310 likely to be cleaved in the gastrointestinal tract due to chemical hydrolases or, more important,
311 microbial activity in the intestine. The activity of chemical hydrolases and intestinal microbes
312 has been demonstrated *in vivo* in swine and *in vitro* using human intestinal microbiota
313 (Berthiller et al. 2011). For this reason, JECFA and EFSA considered to include DON-3-
314 glucoside into the total dietary DON exposure (JECFA 2010).

315

316 4. Conclusion

In conclusion, DON and DON-3-glucoside stability is not affected during baking by the presence of the food additives and the ingredients tested. These additives were tested at the common levels applied in the industry (not necessarily at the maximum allowed concentrations) in different kinds of bakery products, although in this study we used an 'standard' baked analogue. Thus, the tested additives should not be taken into account for the establishment of performance objectives (PO) regarding DON and DON-3-glucoside content. However, more studies should be made with additives used in the food industry using higher concentrations, closer to the maximum level permitted by EU. Moreover, we confirmed that DON and DON-3-glucoside concentrations can vary during heating and that DON-3-glucoside even increases after baking.

5. Acknowledgements

The authors are grateful to the Spanish government (project AGL2014-55379-P) for providing financial support. A. Vidal thanks the Spanish government (Ministry of Education) for the pre-doctoral grant.

6. References

Bergamini E, Catellani D, Dall'Asta C, Galaverna G, Dossena A, Marchelli R, Suman M. 2010. Fate of *Fusarium* mycotoxins in the cereal product supply chain: The deoxynivalenol (DON) case within industrial bread-making technology. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 27:677-687.

338 Berthiller F, Dall'asta C, Corradini R, Marchelli R, Sulyok M, Krska R, Adam G, Schuhmacher
339 R. 2009. Occurrence of deoxynivalenol and its 3-β-D-glucoside in wheat and maize. Food Addit
340 Contam Part A Chem Anal Control Expo Risk Assess. 26:507-511.

341 Berthiller F, Krska R, Domig KJ, Kneifel W, Juge N, Schuhmacher R, Adam G. 2011.
342 Hydrolytic fate of deoxynivalenol-3-glucoside during digestion. Toxicol Lett. 206:264-267.

343 Boyacioglu D, Heitiarachchy NS, D'appolonia BL. 1993. Additives Affect Deoxynivalenol
344 (Vomitoxin) Flour during Breadbaking. J Food Sci. 58:416-418.

345 Bretz M, Beyer M, Cramer B, Knecht A, Humpf HU. 2006. Thermal degradation of the
346 *Fusarium* mycotoxin deoxynivalenol. J Agric Food Chem. 54:6445–6451.

347 Cano-Sancho G, Valle-Algarra FM, Jiménez M, Burdaspal P, Legarda TM, Ramos A.J, Sanchis
348 V, Marín S. 2011. Presence of trichothecenes and co-occurrence in cereal-based food from
349 Catalonia (Spain). Food Control 22:490-495.

350 Cano-Sancho G, Gauchi J-P, Sanchis V, Marín S, Ramos AJ. 2011. Quantitative dietary
351 exposure assessment of the catalonian population (Spain) to the mycotoxin deoxynivalenol.
352 Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 28:1098-1109.

353 Chang J, Li J, Zhao Y, Zhu Y, Ding N, Liu Y. 2015. Degradation effect of deoxynivalenol in
354 bread processing. J Chin Inst Food Sci Technol. 15:199-205.

355 Dall'Asta C, Dall'Erta A, Mantovani P, Massi A, Galaverna G. 2013. Occurrence of
356 deoxynivalenol and deoxynivalenol-3-glucoside in durum wheat. World Mycotoxin J. 6:83-91.

357 De Angelis E, Monaci L, Pascale M, Visconti A. 2013. Fate of deoxynivalenol, T-2 and HT-2
358 toxins and their glucoside conjugates from flour to bread: an investigation by high-performance
359 liquid chromatography high-resolution mass spectrometry. Food Addit Contam Part A Chem
360 Anal Control Expo Risk Assess. 30:345-355.

De Boevre M, Jacxsens L, Lachat C, Eeckhout M, Di Mavungu JD, Audenaert K, Maene P, Haesaert G, Kolsteren P, De Meulenaer B, De Saeger S. 2013. Human exposure to mycotoxins and their masked forms through cereal-based foods in Belgium. Toxicol Lett. 218:281-292.

Desmarchelier A, Seefelder, W. 2010. Survey of deoxynivalenol and deoxynivalenol-3-glucoside in cereal-based products by liquid chromatography electrospray ionization tandem mass spectrometry. World Mycotoxin J. 4:29–35.

Döll S, Dänicke S. 2011. The *Fusarium* toxins deoxynivalenol (DON) and zearalenone (ZON) in animal feeding. Prev Vet Med. 102: 132-145.

[EC] European Commission. 2006. Commission Regulation (EC) No. 1881/2006 of 19/12/2006, setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union, L364, 5e24.

Generotti S, Cirlini M, Malachova A Sulyok M, Berthiller F, Dall'Asta C, Suman M. 2015. Deoxynivalenol & deoxynivalenol-3-glucoside mitigation through bakery production strategies: effective experimental design within industrial rusk-making technology. Toxins 7:2773-2790.

JECFA. 2010. WHO Technical Report Series 959: JECFA, 2010: Seventy-second Report of the Joint FAO/WHO Expert Committee on Food Additives: Evaluation of Certain Contaminants in Food. WHO Technical Report Series. 959.

Kostelanska M, Dzuman Z, Malachova A, Capouchova I, Prokinova E, Skerikova A, Hajslova J. 2011. Effects of milling and baking technologies on levels of deoxynivalenol and its masked form deoxynivalenol-3-glucoside. J Agric Food Chem. 59:9303-9312.

Marín S, Ramos AJ, Cano-Sancho G, Sanchis V. 2013. Mycotoxins: Occurrence, toxicology, and exposure assessment. Food Chem Toxicol. 60:218-237.

384 Mishra S, Dixit S, Dwivedi P D, Pandey H P, Das M. 2014. Influence of temperature and pH
385 on the degradation of deoxynivalenol (DON) in aqueous medium: comparative cytotoxicity of
386 DON and degraded product. Food Addit Contam Part A Chem Anal Control Expo Risk Assess.
387 31:121-131.

388 Neira MS, Pacin AM, Martínez EJ, Moltó G, Resnik SL. 1997. The effects of bakery processing
389 on natural deoxynivalenol contamination. Int J Food Microbiol. 37:21-25.

390 Numanoglu E, Gökmen V, Uygun U, Koksel H. 2012. Thermal degradation of deoxynivalenol
391 during maize bread baking. Food Addit Contam Part A Chem Anal Control Expo Risk Assess.
392 29: 423-430.

393 Poppenberger B, Berthiller F, Lucyshyn D, Siebrer T, Schumacher R, Krska R, Kuchler K,
394 Glössl J, Luschnig C, Adam G. 2003. Detoxification of the *Fusarium* mycotoxin
395 deoxynivalenol by a UDP-glucosyltransferase from *Arabidopsis thaliana*. J Biol Chem.
396 278:47905-47914.

397 Rasmussen RR, Storm IMLD, Rasmussen PH, Smedsgaard J, Nielsen KF. 2010. Multi-
398 mycotoxin analysis of maize silage by LC-MS/MS. Anal Bioanal Chem. 397: 765–776.

399 Rocha O, Ansari K, Doohan FM. 2005. Effects of trichothecene mycotoxins on eukaryotic cells:
400 A review. Food Addit Contam. 22: 369-378.

401 Rotter BA, Prelusky DB, Pestka JJ. 1999. Invited review: Toxicology of deoxynivalenol
402 (vomitoxin). J Toxicol Environ Health Sci. 48: 1-34.

403 [SCF] Scientific Committee on Food. 1999. Opinion on Fusarium Toxins - Part 1:
404 Deoxynivalenol (DON) (expressed on 2 December 1999).

405 SCOOP Reports on Tasks for Scientific Cooperation. 2003. Report of exports participating in
406 Task 3.2.10. Collection of occurrence data of Fusarium toxins in food and assessment of dietary

intake by the population of EU member states. Directorate-General Health and Consumer Protection Brussels Belgium Available from: http://ec.europa.eu/food/fs/scoop/task3210.pdf.

Sewald N, Von Gleissenthall JL, Schuster M, Müller G, Aplin RT. 1992. Structure elucidation of a plant metabolite of 4-desoxynivalenol. Tetrahedron-Asymmetry. 3:953-960.

Simsek S, Burgess K, Whitney KL, Gu Y, Qian SY. 2012. Analysis of deoxynivalenol and deoxynivalenol-3-glucoside in wheat. Food Control. 26:287-292.

Sobrova P, Adam V, Vasatkova A, Beklova, M, Zeman L, Kizek R. 2010. Deoxynivalenol and its toxicity. Interdiscip Toxicol. 3: 94-99.

Stanciu O, Juan C, Miere D, Loghin F, Mañes J. 2017. Occurrence and co-occurrence of *Fusarium* mycotoxins in wheat grains and wheat flour from Romania. Food Control. 73: 147-155.

Suman M, Manzitti A, Catellani D. 2012. A design of experiments approach to studying deoxynivalenol and deoxynivalenol-3-glucoside evolution throughout industrial production of whole grain crackers exploiting LC-MS/MS techniques. World Mycotoxin J. 5:241-249.

Suman M, Bergamini E, Catellani D, Manzitti A. 2013. Development and validation of a liquid chromatography/linear ion trap mass spectrometry method for the quantitative determination of deoxynivalenol-3- glucoside in processed cereal-derived products. Food Chem. 136: 1568-1576.

Vaclavikova M, Malachova A, Veprikova Z, Dzuman Z, Zachariasova M, Hajslova J. 2013. “Emerging” mycotoxins in cereals processing chains: Changes of enniatins during beer and bread making. Food Chem. 136:750-757.

Valle-Algarra FM, Mateo EM, Medina Á, Mateo F, Gimeno-Adelantado JV, Jiménez M. 2009. Changes in ochratoxin A and type B trichothecenes contained in wheat flour during dough

fermentation and bread-baking. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 26:896-906.

Vidal A, Marín S, Ramos AJ, Cano-Sancho G, Sanchis V. 2013. Determination of aflatoxins, deoxynivalenol, ochratoxin A and zearalenone in wheat and oat based bran supplements sold in the Spanish market. Food Chem Toxciol. 53: 133-138.

Vidal A, Morales H, Sanchis V, Ramos AJ, Marín S. 2014. Stability of DON and OTA during the breadmaking process and determination of process and performance criteria. Food Control. 40:234-242.

Vidal A, Marín S, Morales H, Ramos AJ, Sanchis V. 2014. The fate of deoxynivalenol and ochratoxin A during the breadmaking process, effects of sourdough use and bran content. Food Chem Toxicol. 68:53-60.

Vidal A, Sanchis V, Ramos AJ, Marín S. 2015. Thermal stability and kinetics of degradation of deoxynivalenol, deoxynivalenol conjugates and ochratoxin A during baking of wheat bakery products. Food Chem. 178:276-286.

Vidal A, Ambrosio A, Sanchis V, Ramos AJ, Marín S. 2016. Enzyme bread improvers affect the stability of deoxynivalenol and deoxynivalenol-3-glucoside during breadmaking. Food Chem. 208:288-296.

Vidal A, Sanchis V, Ramos AJ, Marín S. 2016. The fate of deoxynivalenol through wheat processing to food products. Curr Opin Food Sci. 11:34-39.

Vidal A, Sanchis V, Ramos AJ, Marín S. 2017. Effect of xylanase and α-amylase on DON and its conjugates during the breadmaking process. Food Res Int. 101: 139-147.

Zachariasova M, Vaclavikova M, Lacina O, Vaclavik L, Hajslova J. 2012. Deoxynivalenol oligoglycosides: New "masked" *Fusarium* toxins occurring in malt, beer, and breadstuff. J Agric Food Chem. 60:9280-9291.

Table 1. List of all additives and additional ingredients tested, with the E identification, concentrations used, pH conditions and the average concentration (µg/kg) ± standard deviation with % of reduction referred to the control for DON (201 ± 28 µg/kg) and DON-3-glucoside (57 ± 11 µg/kg).

Name of additives	E identification	Quantity of added additive/ingredient (%)	pH	DON		DON-3-glucoside	
				Concentration (µg/kg)	Reduction (%)	Concentration (µg/kg)	Reduction (%)
Control	-	-	6.1	201 ± 28	-	57 ± 11	-
Ascorbic acid	E300	0.02	5.2	186 ± 19	7.8	62 ± 16	-7.5
Citric acid	E330	0.30	5.3	196 ± 25	2.9	51 ± 10	11.2
Sorbic acid	E200	0.20	5.3	192 ± 35	4.8	68 ± 10	-19.6
Calcium propionate	E282	0.3	5.4	190 ± 12	5.7	58 ± 19	-0.5
Lecithin	E322	0.40	6.1	204 ± 30	-1.0	60 ± 20	-4.4
Diacetyltartaric acid esters of mono and diglycerides of fatty acids	E472a	0.30	6.0	210 ± 20	-4.1	65 ± 15	-13.3
Calcium phosphate	E341	0.25	5.8	221 ± 32	-9.5	64 ± 26	-11.7
Disodium diphosphate	E450i	0.30	6.2	184 ± 24	8.7	51 ± 8	10.8
Xanthan gum	E415	0.2	6.0	237 ± 12	-17.6	59 ± 26	-3.0
Polydextrose	E1200	26 (sucrose was replaced by polydextrose)	6.1	201 ± 23	0.3	62 ± 13	-7.5
Sorbitol	E420i	3	6.1	209 ± 40	-4.0	61 ± 19	-6.3
Sodium bicarbonate	E500i	1	6.3	238 ± 31	-18.3	56 ± 23	2.8
Gluten of wheat	-	2	6.0	196 ± 20	2.5	65 ± 12	-13.5
Malt flour	-	1	6.0	226 ± 32	-12.2	61 ± 17	-6.3

Table 2. Performance of methods for the determination of DON and deoxynivalenol-3-glucoside in bakery products.

Mycotoxin	Matrix	LOD[a] (µg/kg)	LOQ[b] (µg/kg)	n	Spiking level (µg/kg)	Recovery [c] (%)	RSDr[d](%)
DON	Food matrix	50	150	3	150	108±15	14.2
				3	350	93±5	5.0
				5	750	99±1	1.6
	Flour	60	180	3	300	123 30	41
				3	500	87 9	7
DON-3-glucoside	Flour	14	42	3	50	80±9	12
				3	250	80±5	6
				5	500	67±11	18

[a] LOD = Limit of detection.
[b] LOQ = Limit of quantification.
[c] Mean value ± standard deviation.
[d] RSDr = relative standard deviation.

Figure 1. Recorded temperatures in the oven and in the centre of the baking cakes.